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PURIFIKASI DAN KARAKTERISASI PROTEIN IMUNOGLOBULIN Y ANTI DENGUE SEBAGAI DASAR DALAM PEMBUATAN KIT DIAGNOSTIK DEMAM BERDARAH

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PURIFICATION AND CHARACTERIZATION PROTEIN OF ANTIDENGUE IMUNOGLOBULIN Y ANTIDENGUE AS DIAGNOSTIC KIT FOR DENGUE

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ABSTRACT

Specific antibodies produced in chickens offer several important advantages over producing antibodies in mammals, therefore IgY has been widely used to diagnosis in different diseases. In this study, an approach for large-scale production of Ig Y specific anti-dengue for use as a potential diagnosis for Dengue disease. Lohman laying hens were immunized intramuscularly with dengue virus that had been inactivated using beta-propiolactone. The immunizations were repeated four times with dose of each 80 μg of antigen (viral protein) with an interval of one week. The first immunizations were dengue antigen mixed with Fruend Adjuvant Complete and subsequently mixed with Freund Adjuvant Incomplete. Egg yolk was separated from egg white and immunoglobulin Y (IgY) antibody was then purified by multiple polyethylene glycol (PEG) 6000 extraction and ammonium sulfate purification steps. Antibody response in yolk was detected by agar gel precipitation test (AGPT) and the protein pattern was detected using polyacrilamid gel electrophoresis (SDS-PAGE). Specific activity of the antibody was tested using commercial ELISA and Western blotting. Antibody of dengue was detected and produce a specific line of precipitation in AGPT beginning the second week after the first immunization. Analysis of results obtained with ELISA showed significant increase in the dengue-specific antibodies after two weeks from the primary immunization. Through the effect of boostering; the anti-dengue antibody levels reached a plateau at six four weeks from the primary immunization and remained significantly higher till the end of observation period. SDS-PAGE revealed the IgY preparation to be pure and dissociated into protein bands with molecular weights of 145; 66; 45, 33 and 26 kDa and western blot analysis revealed the presence of anti-dengue IgY in egg yolks protein, with a molecular weights of approximately 66 kDa

These results suggested that chicken IgY could be a practical strategy in large-scale production of Ig Y specific anti-dengue for diagnostic KIT of dengue.

Keyword: Immunoglobulin Y antidengue, Diagnostic KIT